

Therapeutic non-invasive brain stimulation in amyotrophic lateral sclerosis: rationale, methods, and experience

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Keywords: amyotrophic lateral sclerosis, motor neurone disease, non-invasive brain stimulation, transcranial magnetic stimulation, therapeutics

Words: 3588 Tables: 1 Figures: 3 Refs: 86

Abstract

The neurodegenerative syndrome amyotrophic lateral sclerosis (ALS) is characterised by increased cortical excitability, thought to reflect pathological changes in the balance of local excitatory and inhibitory neuronal influences. Non-invasive brain stimulation (NIBS) has been shown to modulate cortical activity, with some protocols showing effects that outlast the stimulation by months. NIBS has been suggested as a potential therapeutic approach for disorders associated with changes in cortical neurophysiology, including ALS. This article reviews NIBS methodology, rationale for its application to ALS and progress to date.

Introduction

Amyotrophic lateral sclerosis (ALS) is an aetiologically complex neurodegenerative syndrome of the motor system and its wider cerebral networks¹. There is a clinicopathological overlap with frontotemporal dementia (FTD) through a shared molecular signature of neuronal and glial cytoplasmic aggregates of the 43 kDa transactive region DNA-binding protein, TDP-43. No highly-effective disease-modifying therapy exists and progressive muscular weakness results in a median survival of 30 months from symptom onset. Understanding the underlying pathological processes is therefore of vital importance.

A number of studies have been performed which have investigated changes in inhibitory signalling in primary motor cortex early in disease. The observation of a somatotopically-enlarged region of cortical activation during the performance of a focal motor task using activation positron emission tomography (PET) led to the first hypothesis that ALS pathogenesis may involve a relative reduction in cortical inhibitory interneuronal signalling², as cortical representations are thought to be determined by local circuits involving the inhibitory neurotransmitter γ -aminobutyric acid (GABA)³.

A substantial body of evidence suggests pathological loss of inhibitory neuronal influences in ALS⁴. Post mortem studies show depletion of GABA-ergic (parvalbumin positive) inhibitory interneurons in the motor cortex in ALS, irrespective of the severity of Betz cell loss⁵. Additionally, reduction in GABA_A-receptor α 1

subunit expression in surviving pyramidal cells of the prefrontal cortex implies specific dysregulation of GABA_A mediated inhibitory signalling⁶. Post mortem findings are corroborated in vivo by evidence of reduced motor cortex binding of the GABA_A-selective PET ligand flumazenil⁷. Cortical GABA-ergic signalling is further implicated by loss of physiological short-interval cortical inhibition (SICI) in ALS - considered to be a GABA_A mediated physiological phenomenon demonstrated by paired-pulse transcranial magnetic stimulation (ppTMS)⁸. Loss of SICI is a consistent observation across the clinically heterogenous syndrome of ALS and pre-dates the onset of symptoms in carriers of ALS-causing genetic variants⁹.

Another manifestation of lost intracortical inhibitory signalling may be more widely dysfunctional cortical dynamics. For example, interhemispheric functional connectivity¹⁰ and callosal structural integrity^{11,12} are reduced in ALS. Furthermore, integrated analysis of white-matter structural information and resting-state functional connectivity suggests a pattern of increased functional connectivity with worsening severity of white-matter tract damage with disease progression^{13,14}. A putative mechanistic explanation for these various lines of evidence is a progressive pathological loss of cortical inhibitory signalling over time.

In addition to cortical dysfunction, loss of inhibition may contribute more directly to the degenerative process in ALS. Excitotoxic mechanisms of cell death have been postulated in relation to excess glutamatergic activity in ALS¹⁵. The first licensed disease-modifying

treatment for ALS, albeit with only a small effect on survival, is the broadly anti-excitatory drug riluzole^{16,17}. Although several drugs with broadly pro-inhibitory cortical action have appeared ineffective for the treatment of ALS, such studies relied on relatively insensitive clinical outcome measures such as manual muscle strength testing and rate of decline of a composite disability score¹⁸.

Modulation of cortical inhibitory influences in ALS is therefore a plausible strategy to mitigate disrupted motor system function, and more speculatively, to modify the degenerative process.

Non-invasive brain stimulation (NIBS) encompasses a set of promising research tools that may modulate cortical inhibitory signalling in this way¹⁹. NIBS approaches are applied through the scalp and skull with the common goal of modulating brain activity using some form of energy - electrical current, magnetic pulses or focused ultrasound. These operate at a wide range of scales, from cortical microcircuit to wider brain network, and can exert effects that persist for hours to days (**Figure 1**). However, there remain significant doubts about the efficacy and reproducibility of NIBS in even the healthy brain.

This article reviews the range of NIBS techniques, underlying physiological mechanisms, current limitations and potential application to ALS.

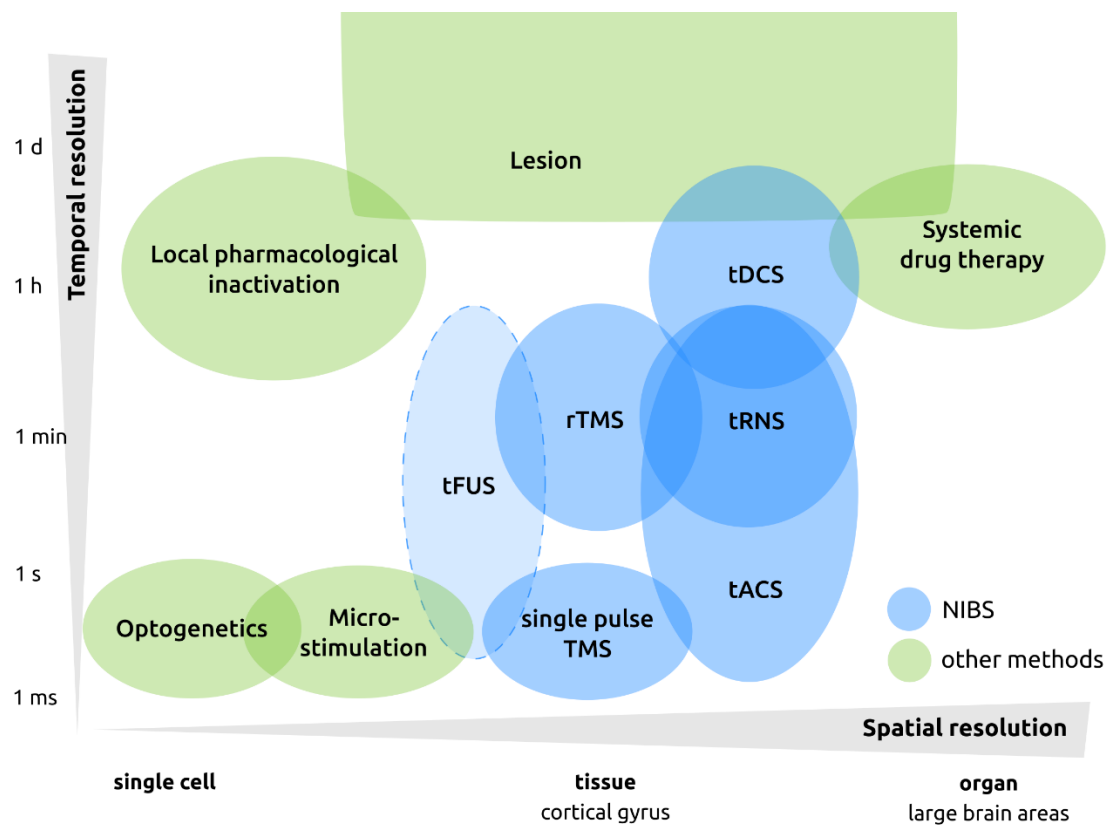


Figure 1: A simplified overview of a range of interventions that alter brain function, with their spatial and temporal scale. tFUS is very early in development, putatively offering finer spatial resolution than other NIBS techniques. Adapted with permission from Polania 2018, Nature Reviews Neuroscience²⁰.

Stimulation methods

Historical development

Electrical stimulation of biological tissues has a long history. The Roman physician Scribonius Largus reportedly applied electric shocks from live torpedo fish to the forehead in the treatment of headache. Galvani demonstrated in 1791 that electrical current could make a dismembered frog leg twitch. Ten years later, his nephew Aldini applied direct-current stimulation over a moistened area of the parietal scalp in a farmer diagnosed with "melancholy", reporting improvement in mood²¹. A variety of enterprising practitioners from 1870-1930 promoted do-it-yourself electrical therapies for indications including headache, pain, insomnia and low mood²². These efforts led to the development in 1938 of electroconvulsive therapy (ECT) by Italian neurologists Ugo Cerletti and Luigi Bini²³. The original forms of ECT involved the application of much larger currents across the entire head to evoke seizures, and supplanted interest in the lower currents used for transcranial stimulation for decades. The promise of lower currents as potential therapies has only recently been more widely investigated.

NIBS approaches are commonly categorised according to the form of energy used to stimulate the brain: magnetic, electrical (direct or alternating current) and, most recently, ultrasonic (**Figure 2**).

Transcranial electrical stimulation can be further subdivided into transcranial direct current (tDCS), alternating current (tACS) and random noise stimulation (tRNS). NIBS can also be classified into

neurostimulatory approaches, whereby stimulation directly induces action potentials in the underlying neuronal tissue, or neuromodulatory techniques, which act by modulating the ongoing firing rate of stimulated tissue. In both categories, a range of secondary mechanisms have been proposed to discuss the long term effect - this is discussed further for each modality.

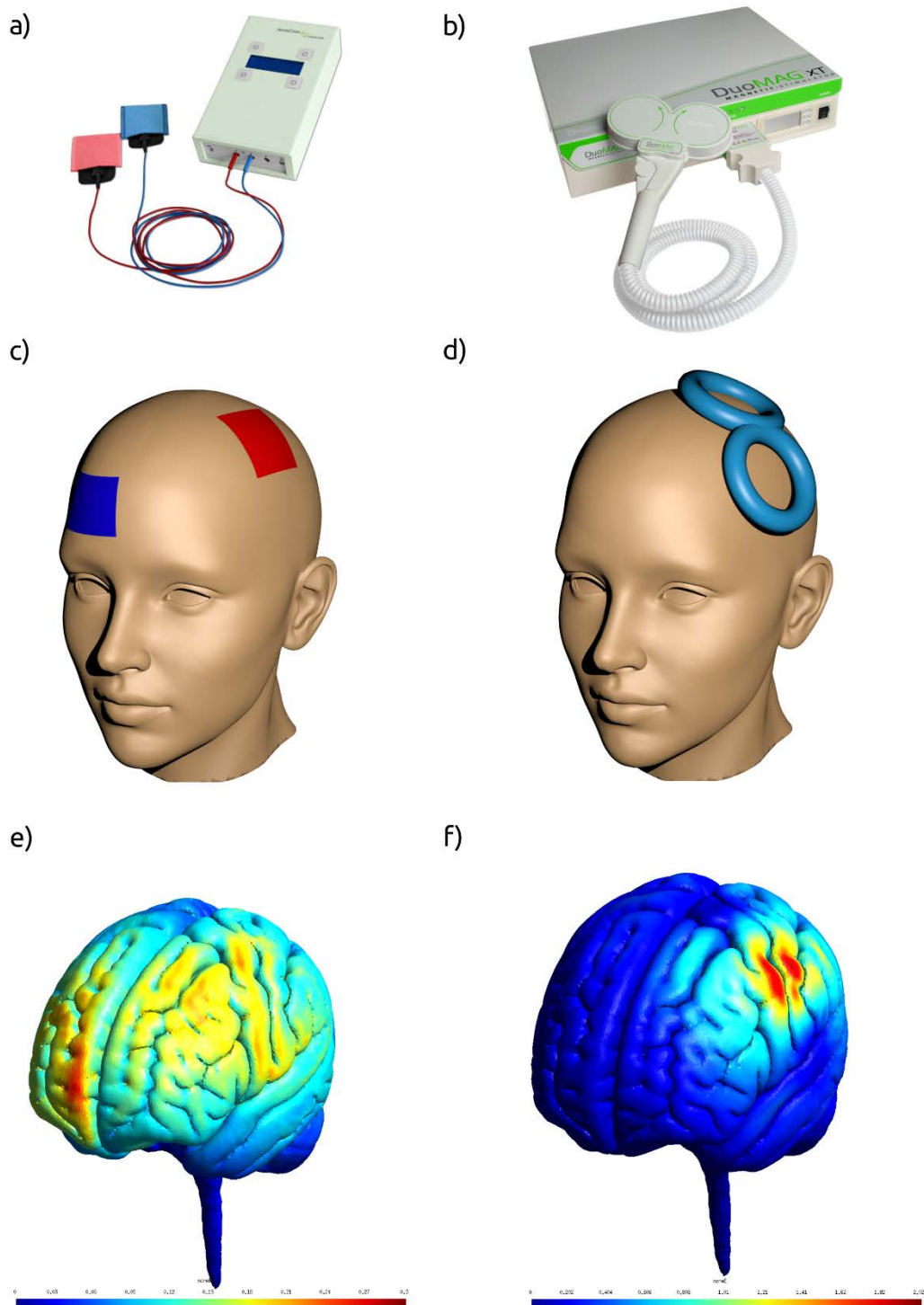


Figure 2. Examples of commercial (a) TDCS and (b) TMS equipment (c,d) coil/electrode montage over motor cortex and (d,e) maps of electrical fields generated.

Transcranial direct current stimulation

Modern interest in transcranial direct current electrical stimulation was sparked by the demonstration that weak direct current stimulation over the motor cortex modulated excitability in the cortical representation of the hand²⁴. tDCS involves the application of a small direct current (1-2 mA) through a circuit formed by two or more electrodes (at least one cathode and anode) applied to the scalp with conductive gel or paste. This produces a gradient electric field that alters resting neuronal membrane potential and excitability properties presumed to be subthreshold for action potential generation^{25,26}. Protocols vary extensively in electrode size, placement, current applied and duration of stimulation^{27,28}.

Depending on the protocol used, the effect of a single session of tDCS on cortical excitability can persist for 90 minutes beyond cessation of stimulation, or longer²⁹. The frequency of tDCS sessions is also thought to determine the duration of effect. Repeated sessions with short intervening breaks are associated with excitability alterations up to 24 hours later³⁰, and longer-term behavioural effects have been demonstrated³¹.

Wide inter-individual and between-session is reported in tDCS. This may be partly explained by the underlying mechanisms of tDCS action. In addition to human studies, cell, animal and computational models have

been used to elucidate the physiological mechanisms of tDCS. Brain tissue preparations can model the effect of an electrical field at the single cell level, with complex effects depending on the predominant orientation of the cell relative to the field (see Figure 3a). Where cellular structures that originate action potentials (soma, axon hillock, and apical dendrites)³² are aligned with the field direction, the cell becomes electrically polarised – depolarised at the soma and hyperpolarised at the axon terminal³³. This reduces the threshold potential at the axon hillock. However, where a cell is perpendicular to the field, this effect is negligible as smaller compartments bounded by membranes restrict current and the gradient is smaller³⁴. As a result, cortical microarchitecture, macroscopic folding, and orientation relative to the conducting skin pad will all determine which sub-populations of neurons become more excitable. Computational models of the electric field also demonstrate the impact of pad size, skin contact, skull thickness, and head shape on the field geometry²⁶. The acute effects of tDCS on excitability are abolished by voltage-gated ion channel blockade, but unaffected by glutamate or GABA antagonism, suggesting the primacy of electrical effects in the acute phase³⁵. However, in the same study, the NMDA-receptor antagonist dextromethorphan abolished the after-effect of both anodal and cathodal tDCS, suggesting a role for glutamatergic synaptic function. The NMDA-receptor is known to be a non-specific cation channel.

. Increased glutamatergic activity may increase excitability after stimulation ends through repeated calcium influx through the NMDA

receptor. This drives the transcriptional changes of long term potentiation (LTP), an intracellular calcium driven process³⁶. Neurochemical changes after stimulation can also be quantified using MR spectroscopy, suggesting increased glutamate after anodal stimulation and decreased glutamate after cathodal stimulation, with decreased GABA in both cases^{37,38}. Motor cortex GABA is also significantly reduced 1 hour after anodal tDCS³⁹ – a role has been proposed for GABA as a gating factor whose reduction allows plastic changes to occur. The neurochemical basis of the tDCS after-effect helps explain its variability with alertness, sleep quality, caffeine, and between individuals.

Though less well understood, tDCS may also influence brain function at larger scales. Motor cortex (M1) anodal tDCS results in increased functional connectivity for both long-range connections within M1⁴⁰, and those between M1 other motor-related brain regions⁴¹.

Other electrical stimulation

A sinusoidal, or alternating, current is delivered across two scalp electrodes (of similar size and placement to tDCS) in transcranial alternating current stimulation (tACS). tACS is believed to interact with ongoing frequency-specific activity within the stimulated brain regions, potentially leading to entrainment of endogenous rhythms, though this has yet to be definitively proven. The behavioural effects of tACS are modest, though with increasingly sophisticated waveform

parameters, there is increasing evidence as to its behavioural relevance^{42,43}.

High frequency (100 - 640Hz) transcranial random noise stimulation (trNS) with a random stimulation pattern with a bell-shaped probability distribution has also been suggested to increase motor cortex excitability⁴⁴. The physiological basis, reproducibility, and effect size of tACS/trNS is less well understood than tDCS and to date, they have not been applied to ALS.

Transcranial ultrasound

In 1955, the formation of a pinpoint brain lesion using high energy focused ultrasound was demonstrated in a cat⁴⁵. Precise intracranial lesioning using mechanical energy has been widely applied as a non-invasive neurosurgical technique in movement disorders and malignancy. At much lower energies where tissue is not damaged, a neuromodulatory role for transcranial focused ultrasound (tFUS) is emerging. Application of tFUS over the primary somatosensory cortex can elicit tactile sensations in the hand⁴⁶. Recently, a neuromodulatory effect on motor cortex excitability has been suggested when tFUS is applied in conjunction with TMS⁴⁷. Focused ultrasound is in its very early days as a neuromodulatory technique and its effects on the brain are not yet clear. As this technique is refined and the underlying mechanisms investigated, it may find wider application, with key advantages of precise spatial localisation and access to deep structures.

Transcranial magnetic stimulation

Magnetic fields have historically been shown to interact with nervous system function. The French physician Jacques Arsène d'Arsonval reported in 1896 that on placing his head inside a solenoid coil producing a magnetic field he experienced phosphenes and vertigo.

In 1985, Anthony Barker and colleagues at the University of Sheffield reported using a pulsed magnetic field to stimulate the motor cortex and peripheral nerves⁴⁸. Modern TMS remains fundamentally similar - involving the use of a magnetic field generator ("coil") placed over the scalp. Coils vary in material and geometry, resulting in differing magnetic field patterns e.g. 'figure of eight' coils have been developed to deliver a more focal pattern of activation than the original round coils⁴⁹. A short-lived, large, electric current is passed through the hand-held coil, which generates a magnetic field. Through electromagnetic induction, this rapidly-changing magnetic field induces a current flowing parallel but opposite to that in the coil in the brain, a nearby conducting medium. The current produced can be targeted to the hand area of the motor cortex using electromyographic recording of the motor evoked potential (MEP) in the intrinsic muscles (reviewed in detail⁵⁰).

As with tDCS, the effects of TMS vary depending on the protocol used. Single pulse and paired-pulse protocols are powerful neurophysiological tools to investigate cortical excitability. The

paired-pulse TMS measures intra-cortical facilitation (ICF) and short-interval cortical inhibition (SICI) measure aspects of cortical glutamatergic and GABA-ergic activity respectively⁵¹. TMS can also be used as a tool for modulating cortical excitability. Repetitive TMS (rTMS), where trains of pulses are used at a variety of frequencies, has been suggested to have prolonged effects on cortical excitability. The specific frequency and pattern of stimulation can produce divergent responses – low frequency constant-rate rTMS (1Hz) decreases motor cortex excitability, while excitability increases with higher stimulation frequencies (10Hz)⁵².

More recently, higher frequency TMS stimulation patterns that mimic physiologically occurring activity have been developed. Similar responses to constant-rate stimulation have been reported with much shorter stimulation time and fewer pulses. Theta burst stimulation (TBS) is an increasingly-used pattern⁵³. The theta burst is a train of three closely spaced pulses at 50Hz which is delivered every 200ms. This pattern can be delivered as continuous TBS (cTBS) as a 40 second train with a total of 600 pulses, or as intermittent TBS (iTBS), with 2 second blocks of TBS separated by 8 second intervals with no stimulation (**Figure 3**). cTBS protocols have been suggested to produce depression of motor cortex excitability for up to 60 minutes post-stimulation⁵³, but with more recent debate as to whether effects are reproducible in all subjects⁵⁴.

The variability (between-protocol, inter-individual and intra-individual) of rTMS effects may be explained by individual-level factors (age, sex, genetics, sleep quality, exposure to caffeine) and

tissue-level factors affecting the specific populations of cortical neurons stimulated. As with tDCS, the orientation of cortical folding with respect to the TMS coil will affect the direction and depth of currents induced in the brain⁴⁹. Cortical cytoarchitecture, axon fibre diameter and preferential axon orientation will affect which specific populations of neurons are stimulated. In particular, inter-individual variation in intracortical circuit function (as measured by latency of MEP responses) can predict response to iTBS versus cTBS⁵⁵.

Neurobiological mechanisms underlying the NIBS after-effect

As with tDCS, mechanisms of synaptic plasticity such as LTP (or the related long term depression [LTD]) have been invoked to explain the rTMS after-effect^{56,57}. NMDA receptor antagonists inhibit the after-effects of both cTBS and iTBS [Huang 2007], suggesting a role for LTP mediated by glutamatergic signalling. The divergent effects of cTBS and iTBS may in part be explained by interactions between GABA-ergic signalling and the mechanisms of LTP, with motor cortex GABA significantly increased after cTBS⁵⁸.

A wide range of other effector mechanisms have been suggested for both tDCS and rTMS, including serotonergic⁵⁹ or dopaminergic⁶⁰ neurotransmission, brain-derived neurotrophic factor, neuroinflammation and glial signalling. A full understanding of NIBS techniques may require the integration of processes across a wide range of spatial and temporal scales and may allow fine tuning of stimulus frequency, intensity and duration to maximise effect.

Figure 3: (a) Representations of electrical and magnetic fields induced by tDCS and TMS with influence of cortical orientation on fields experienced. (b) Different patterns of current that may be applied transcranially. (c) Different stimulation frequencies and the cTBS pattern in repetitive TMS.

Current state of NIBS in ALS

To assess the progress to date in applying NIBS techniques to ALS, a systematic search of the NCBI MEDLINE database was undertaken on 22 February 2019. Entries were included if a term identifying ALS and a term identifying a stimulation technique were present in MeSH terms or Title/Abstract. The full search entries were:

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((Amyotrophic Lateral Sclerosis[MeSH Terms]) OR Motor Neuron Disease[MeSH Terms])) AND ((Transcranial Direct Current Stimulation[MeSH Terms]) OR (Transcranial Magnetic Stimulation[MeSH Terms]) OR (Magnetic Field Therapy[MeSH Terms]))
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(amyotrophic lateral sclerosis[Title/Abstract]) AND ((transcranial direct current stimulation[Title/Abstract]) OR (transcranial magnetic stimulation[Title/Abstract]) OR (transcranial alternating current stimulation[Title/Abstract]) OR transcranial focused ultrasound[Title/Abstract]))
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From 276 initial results, 72 duplicates were removed. The remaining 204 were manually reviewed and filtered for relevance. Results were excluded if not original research (n=55), not conducted in humans with ALS (n=19) or applied stimulation for purposes other than persistent neuromodulation (n=118) - for example paired-pulse TMS as a diagnostic marker. One study was reported twice - the preliminary report was excluded. After review, the eleven remaining studies are summarised (see Table 1).

rTMS

Four studies [references] applied the cTBS pattern. Stimulation frequency varied in the remaining studies. Two studies^{61,62} compared low (1Hz) and high (20Hz) frequency rTMS. Zanette et. al.⁶³ used an intermediate frequency of 5Hz with the stated rationale of combining the effects of reduced cortical excitability with low frequency rTMS and BDNF release at high frequencies.

No consistent pattern in outcomes was seen. Di Lazzaro et. al. reported a statistically significant reduction in the revised ALS Functional Rating Score (ALSFRS-R) decline with active stimulation⁶⁴, but this was not replicated in the follow-up study by the same group in 2009⁶⁵. Munneke et. al. assessed neurophysiological outcomes of a short cTBS protocol, finding that it induced a reduction in MEP in both controls and participants with ALS, but with more sessions of stimulation required to reach the same effect size in the ALS group⁶⁶.

tDCS

Three studies of tDCS in ALS were identified. Quartarone et. al. reported that the after-effects of tDCS seen in healthy individuals were not reproducible in patients with ALS⁶⁷. Munneke et. al. assessed neurophysiological outcomes (single pulse MEP, paired pulse SICI, and ICF) in participants with ALS and in healthy controls after single tDCS sessions lasting 7, 11 or 15 minutes⁶⁸[50](#). The reduction in MEP with tDCS seen in the control group was not replicated in individuals with ALS. No significant effect of tDCS on SICI or ICF was demonstrated in either group. Madhavan et. al. applied anodal, cathodal and sham tDCS over the motor cortex in a single subject safety pilot, with no reported adverse effects⁶⁹. While electrode montages were consistent between studies, the application of 2mA current raises concerns about blinding, while the number and duration of sessions differed significantly.

Current limitations

Small sample sizes, clinical heterogeneity, variable sham techniques and stimulation protocols are all sources of potential type II error in published studies of NIBS in ALS. Power calculations are not routinely presented in the literature⁷⁰ and estimates of the effect size of tDCS in particular have decreased as the body of literature

has grown⁷¹. Where sham stimulation is used at all, it has been suggested that double blind procedures are imperfect⁷², with skin redness in tDCS⁷³ and subtler cues such as the position of the operator⁷⁴ reducing blinding efficacy. TMS sham coils, while mimicking the click produced on pulse generation, cannot reproduce somatosensory stimulation from peripheral nerve stimulation, or indeed motor stimulation, and may be fundamentally insufficient⁷⁵. Adopting more robust operator procedures and publishing indices of blinding efficacy (e.g. Bang's blinding index⁷⁶) may address widespread concerns over blinding methodology in NIBS. Methodological variations may arise from stimulation frequency and duration, along with coil and electrode positioning. There is evidence of non-linear (and sometimes contradictory) responses to increased duration or intensity of tDCS⁷⁷, so that simplistic concepts of pro-inhibitory stimulation become less convincing. Potential drug effects may be ethically difficult to exclude in patient populations, and riluzole has been shown to influence cortical excitability in several studies^{78,79}.

Future prospects

The continued pursuit of NIBS as a therapeutic intervention in ALS is justified based upon the emerging understanding of the pathology as a more complex degeneration across cerebral networks⁸⁰. Neuromodulation via brain stimulation could potentially reduce pathological processes directly or support compensatory mechanisms. Measuring effect is a challenging aspect of all therapeutic studies in ALS. The rate of change in the revised ALS Functional Rating Score (ALSFRS-R) is

clinically relevant but too insensitive to demonstrate short-term proof-of-principle effects.

Progress in NIBS will depend upon greater standardisation of delivery and more robust short and longer-term markers of successful modulation of cortical function. The effects of modulation by NIBS are currently difficult to predict even in the healthy state, given the currently superficial understanding of brain function at the synaptic through to network level. As a more integrated and detailed understanding of the mechanisms of NIBS is built, this may allow more rational design of stimulation protocols to maximise effect. Much as the theta burst stimulation pattern increases efficacy and efficiency of rTMS, protocols with varying frequency and incorporating bursting activity⁸¹ may advance the state of transcranial electrical stimulation. This will be essential to any ambition NIBS as a neuromodulatory or neuroprotective approach to neurodegenerative disease. In the context of ALS, despite underwhelming outcomes to date, advances in the understanding of cortical microcircuit and network level dysfunction^{10,82} point towards therapeutic potential. Alterations in resting-state fMRI of carriers of highly-penetrant ALS-causing genetic mutations are demonstrable prior to the onset of symptoms⁸³. Magnetoencephalography may be sufficiently sensitive to changes in dynamic brain function to detect dysfunction amenable to early modulation⁸⁴. As a more detailed understanding of the complex cortical oscillatory dysfunction in ALS emerges⁸⁵, more sensitive measures of target engagement will be developed⁸⁶. Standardisation of stimulation protocols, and a robust study design that allows for rapidly-accruing

disability, will realise the goal of NIBS as a therapeutic tool in ALS.

References

- 1 Talbot, K., Feneberg, E., Scaber, J., Thompson, A. G. & Turner, M. R. Amyotrophic lateral sclerosis: the complex path to precision medicine. *Journal of neurology* **265**, 2454-2462, doi:10.1007/s00415-018-8983-8 (2018).
- 2 Kew, J. J. *et al.* Cortical function in amyotrophic lateral sclerosis. A positron emission tomography study. *Brain : a journal of neurology* **116 (Pt 3)**, 655-680 (1993).
- 3 Kolasinski, J. *et al.* A Mechanistic Link from GABA to Cortical Architecture and Perception. *Current biology : CB* **27**, 1685-1691 e1683, doi:10.1016/j.cub.2017.04.055 (2017).
- 4 Turner, M. R. & Kiernan, M. C. Does interneuronal dysfunction contribute to neurodegeneration in amyotrophic lateral sclerosis? *Amyotrophic lateral sclerosis : official publication of the World Federation of Neurology Research Group on Motor Neuron Diseases* **13**, 245-250, doi:10.3109/17482968.2011.636050 (2012).
- 5 Nihei, K., McKee, A. C. & Kowall, N. W. Patterns of neuronal degeneration in the motor cortex of amyotrophic lateral sclerosis patients. *Acta neuropathologica* **86**, 55-64 (1993).
- 6 Petri, S. *et al.* GABA(A)-receptor mRNA expression in the prefrontal and temporal cortex of ALS patients. *Journal of the neurological sciences* **250**, 124-132, doi:10.1016/j.jns.2006.08.005 (2006).
- 7 Turner, M. R. *et al.* Distinct cerebral lesions in sporadic and 'D90A' SOD1 ALS: studies with [11C]flumazenil PET. *Brain : a journal of neurology* **128**, 1323-1329, doi:10.1093/brain/awh509 (2005).
- 8 Menon, P. *et al.* Sensitivity and specificity of threshold tracking transcranial magnetic stimulation for diagnosis of amyotrophic lateral sclerosis: a prospective study. *The Lancet. Neurology* **14**, 478-484, doi:10.1016/S1474-4422(15)00014-9 (2015).
- 9 Vucic, S., Nicholson, G. A. & Kiernan, M. C. Cortical hyperexcitability may precede the onset of familial amyotrophic lateral sclerosis. *Brain : a journal of neurology* **131**, 1540-1550, doi:10.1093/brain/awn071 (2008).
- 10 Proudfoot, M. *et al.* Impaired corticomuscular and interhemispheric cortical beta oscillation coupling in amyotrophic lateral sclerosis. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology* **129**, 1479-1489, doi:10.1016/j.clinph.2018.03.019 (2018).
- 11 Chapman, M. C., Jelsone-Swain, L., Johnson, T. D., Gruis, K. L. & Welsh, R. C. Diffusion tensor MRI of the corpus callosum in amyotrophic lateral sclerosis. *Journal of magnetic resonance imaging : JMRI* **39**, 641-647, doi:10.1002/jmri.24218 (2014).

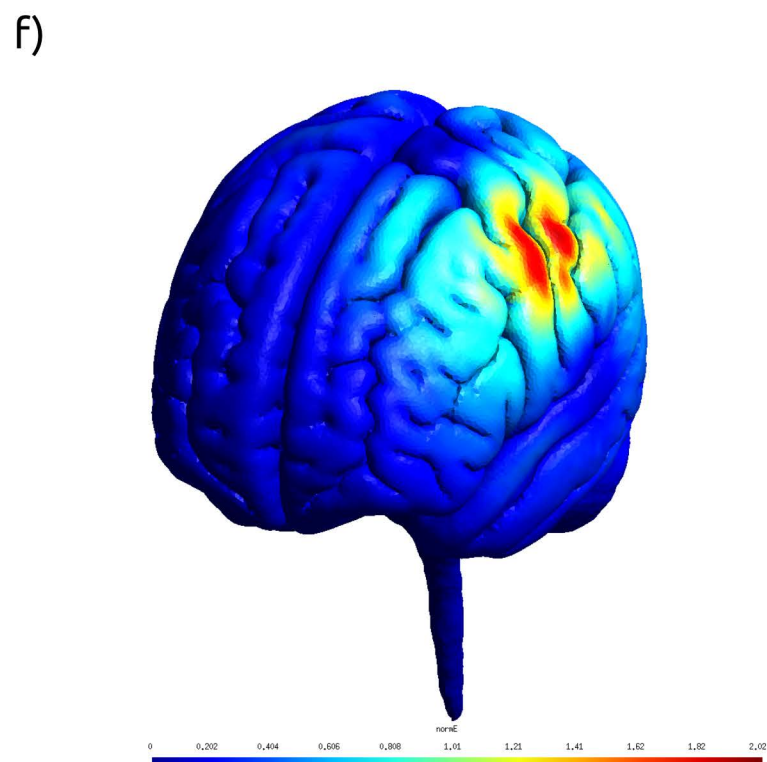
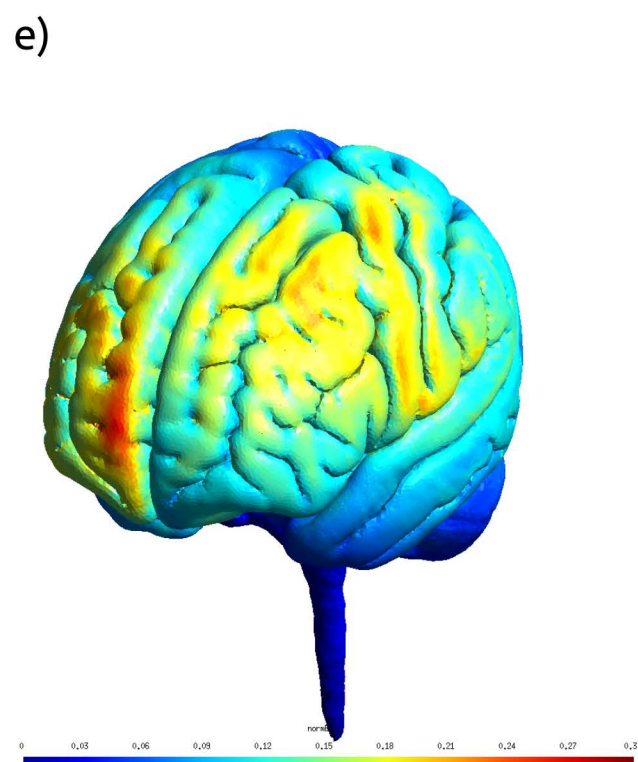
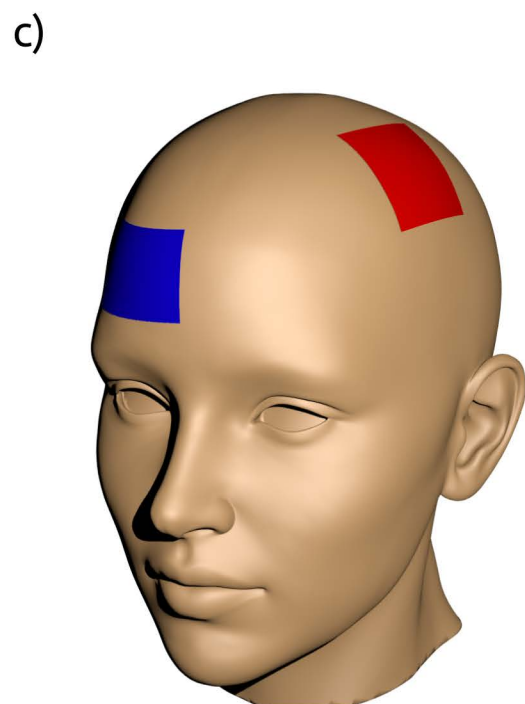
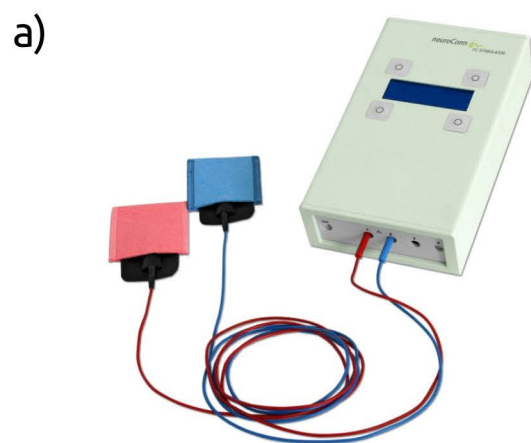
- 12 Filippini, N. *et al.* Corpus callosum involvement is a consistent feature of amyotrophic lateral sclerosis. *Neurology* **75**, 1645-1652, doi:10.1212/WNL.0b013e3181fb84d1 (2010).
- 13 Douaud, G., Filippini, N., Knight, S., Talbot, K. & Turner, M. R. Integration of structural and functional magnetic resonance imaging in amyotrophic lateral sclerosis. *Brain : a journal of neurology* **134**, 3470-3479, doi:10.1093/brain/awr279 (2011).
- 14 Pierpaolo, S. *et al.* Brain functional networks become more connected as amyotrophic lateral sclerosis progresses: A source level magnetoencephalographic study. *NeuroImage: Clinical* (2018).
- 15 Van Den Bosch, L., Van Damme, P., Bogaert, E. & Robberecht, W. The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis. *Biochimica et biophysica acta* **1762**, 1068-1082, doi:10.1016/j.bbadis.2006.05.002 (2006).
- 16 Cheah, B. C., Vucic, S., Krishnan, A. V. & Kiernan, M. C. Riluzole, neuroprotection and amyotrophic lateral sclerosis. *Current medicinal chemistry* **17**, 1942-1199 (2010).
- 17 Doble, A. The pharmacology and mechanism of action of riluzole. *Neurology* **47**, S233-241 (1996).
- 18 Turner, M. R., Parton, M. J. & Leigh, P. N. Clinical trials in ALS: an overview. *Seminars in neurology* **21**, 167-175, doi:10.1055/s-2001-15262 (2001).
- 19 Benali, A. *et al.* Theta-burst transcranial magnetic stimulation alters cortical inhibition. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **31**, 1193-1203, doi:10.1523/JNEUROSCI.1379-10.2011 (2011).
- 20 Polania, R., Nitsche, M. A. & Ruff, C. C. Studying and modifying brain function with non-invasive brain stimulation. *Nature neuroscience* **21**, 174-187, doi:10.1038/s41593-017-0054-4 (2018).
- 21 Parent, A. Giovanni Aldini: from animal electricity to human brain stimulation. *The Canadian journal of neurological sciences. Le journal canadien des sciences neurologiques* **31**, 576-584 (2004).
- 22 Wexler, A. Recurrent themes in the history of the home use of electrical stimulation: Transcranial direct current stimulation (tDCS) and the medical battery (1870-1920). *Brain stimulation* **10**, 187-195, doi:10.1016/j.brs.2016.11.081 (2017).
- 23 Endler, N. S. The Origins of Electroconvulsive Therapy (ECT). *Convulsive therapy* **4**, 5-23 (1988).
- 24 Nitsche, M. A. & Paulus, W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *The Journal of physiology* **527 Pt 3**, 633-639 (2000).
- 25 Stagg, C. J. & Nitsche, M. A. Physiological basis of transcranial direct current stimulation. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry* **17**, 37-53, doi:10.1177/1073858410386614 (2011).
- 26 Opitz, A., Paulus, W., Will, S., Antunes, A. & Thielscher, A. Determinants of the electric field during transcranial direct current stimulation. *NeuroImage* **109**, 140-150, doi:10.1016/j.neuroimage.2015.01.033 (2015).
- 27 Datta, A. *et al.* Gyri-precise head model of transcranial direct current stimulation: improved spatial focality using a ring electrode versus conventional rectangular pad. *Brain stimulation* **2**, 201-207, 207 e201, doi:10.1016/j.brs.2009.03.005 (2009).
- 28 Paulus, W. Transcranial electrical stimulation (tES - tDCS; tRNS, tACS) methods. *Neuropsychological rehabilitation* **21**, 602-617, doi:10.1080/09602011.2011.557292 (2011).

- 29 Nitsche, M. A. & Paulus, W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology* **57**, 1899-1901 (2001).
- 30 Monte-Silva, K. *et al.* Induction of late LTP-like plasticity in the human motor cortex by repeated non-invasive brain stimulation. *Brain stimulation* **6**, 424-432, doi:10.1016/j.brs.2012.04.011 (2013).
- 31 Stagg, C. J. *et al.* Cortical activation changes underlying stimulation-induced behavioural gains in chronic stroke. *Brain : a journal of neurology* **135**, 276-284, doi:10.1093/brain/awr313 (2012).
- 32 Bindman, L. J., Lippold, O. C. & Redfearn, J. W. The Action of Brief Polarizing Currents on the Cerebral Cortex of the Rat (1) during Current Flow and (2) in the Production of Long-Lasting after-Effects. *The Journal of physiology* **172**, 369-382 (1964).
- 33 Jefferys, J. G. Influence of electric fields on the excitability of granule cells in guinea-pig hippocampal slices. *The Journal of physiology* **319**, 143-152 (1981).
- 34 Bikson, M. *et al.* Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices in vitro. *The Journal of physiology* **557**, 175-190, doi:10.1113/jphysiol.2003.055772 (2004).
- 35 Nitsche, M. A. *et al.* Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. *The Journal of physiology* **553**, 293-301, doi:10.1113/jphysiol.2003.049916 (2003).
- 36 Lynch, G., Larson, J., Kelso, S., Barrionuevo, G. & Schottler, F. Intracellular injections of EGTA block induction of hippocampal long-term potentiation. *Nature* **305**, 719-721 (1983).
- 37 Stagg, C. J. *et al.* Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **29**, 5202-5206, doi:10.1523/JNEUROSCI.4432-08.2009 (2009).
- 38 Clark, V. P., Coffman, B. A., Trumbo, M. C. & Gasparovic, C. Transcranial direct current stimulation (tDCS) produces localized and specific alterations in neurochemistry: a (1)H magnetic resonance spectroscopy study. *Neuroscience letters* **500**, 67-71, doi:10.1016/j.neulet.2011.05.244 (2011).
- 39 Patel, H. J. *et al.* Proton Magnetic Resonance Spectroscopy of the motor cortex reveals long term GABA change following anodal Transcranial Direct Current Stimulation. *Scientific reports* **9**, 2807, doi:10.1038/s41598-019-39262-7 (2019).
- 40 Polania, R., Paulus, W. & Nitsche, M. A. Reorganizing the intrinsic functional architecture of the human primary motor cortex during rest with non-invasive cortical stimulation. *PloS one* **7**, e30971, doi:10.1371/journal.pone.0030971 (2012).
- 41 Polania, R., Nitsche, M. A. & Paulus, W. Modulating functional connectivity patterns and topological functional organization of the human brain with transcranial direct current stimulation. *Human brain mapping* **32**, 1236-1249, doi:10.1002/hbm.21104 (2011).
- 42 Nowak, M. *et al.* Driving Human Motor Cortical Oscillations Leads to Behaviorally Relevant Changes in Local GABA Inhibition: A tACS-TMS Study. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **37**, 4481-4492, doi:10.1523/JNEUROSCI.0098-17.2017 (2017).
- 43 Zaehle, T., Rach, S. & Herrmann, C. S. Transcranial alternating current stimulation enhances individual alpha activity in human EEG. *PloS one* **5**, e13766, doi:10.1371/journal.pone.0013766 (2010).

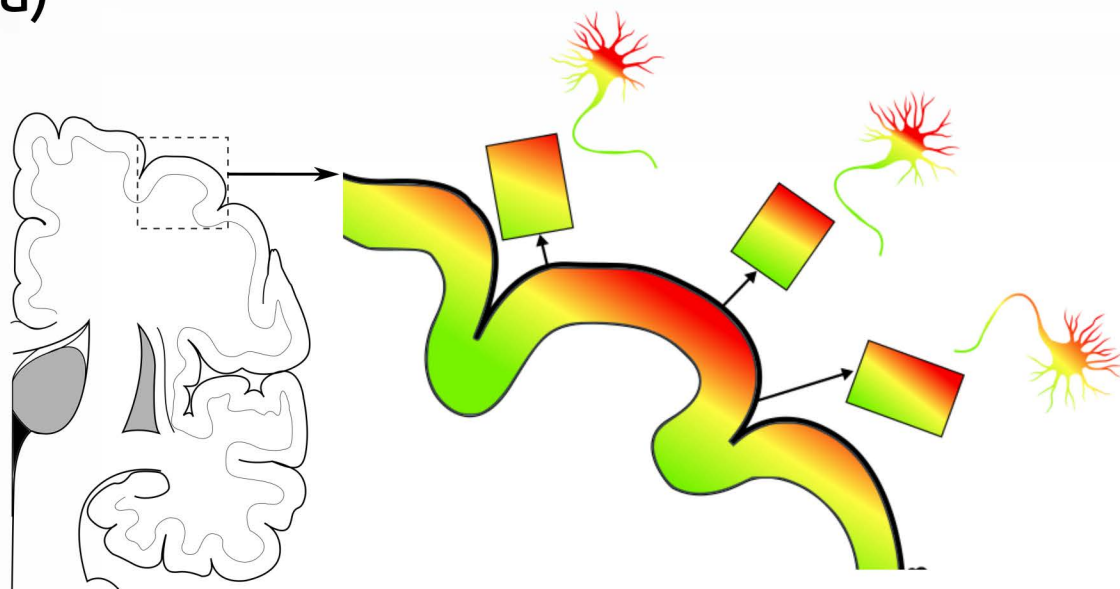
- 44 Terney, D., Chaieb, L., Moliadze, V., Antal, A. & Paulus, W. Increasing human brain excitability by transcranial high-frequency random noise stimulation. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **28**, 14147-14155, doi:10.1523/JNEUROSCI.4248-08.2008 (2008).
- 45 Fry, W. J., Barnard, J. W., Fry, E. J., Krumins, R. F. & Brennan, J. F. Ultrasonic lesions in the mammalian central nervous system. *Science* **122**, 517-518 (1955).
- 46 Lee, W. *et al.* Image-guided transcranial focused ultrasound stimulates human primary somatosensory cortex. *Scientific reports* **5**, 8743, doi:10.1038/srep08743 (2015).
- 47 Legon, W., Bansal, P., Tyshynsky, R., Ai, L. & Mueller, J. K. Transcranial focused ultrasound neuromodulation of the human primary motor cortex. *Scientific reports* **8**, 10007, doi:10.1038/s41598-018-28320-1 (2018).
- 48 Barker, A. T., Jalinous, R. & Freeston, I. L. Non-invasive magnetic stimulation of human motor cortex. *Lancet* **1**, 1106-1107 (1985).
- 49 Deng, Z. D., Lisanby, S. H. & Peterchev, A. V. Electric field depth-focality tradeoff in transcranial magnetic stimulation: simulation comparison of 50 coil designs. *Brain stimulation* **6**, 1-13, doi:10.1016/j.brs.2012.02.005 (2013).
- 50 Hallett, M. Transcranial magnetic stimulation: a primer. *Neuron* **55**, 187-199, doi:10.1016/j.neuron.2007.06.026 (2007).
- 51 Pascual-Leone, A. *et al.* Study and modulation of human cortical excitability with transcranial magnetic stimulation. *Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society* **15**, 333-343 (1998).
- 52 Pascual-Leone, A., Valls-Sole, J., Wassermann, E. M. & Hallett, M. Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. *Brain : a journal of neurology* **117** (Pt 4), 847-858 (1994).
- 53 Huang, Y. Z., Edwards, M. J., Rounis, E., Bhatia, K. P. & Rothwell, J. C. Theta burst stimulation of the human motor cortex. *Neuron* **45**, 201-206, doi:10.1016/j.neuron.2004.12.033 (2005).
- 54 Doeltgen, S. H. & Ridding, M. C. Low-intensity, short-interval theta burst stimulation modulates excitatory but not inhibitory motor networks. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology* **122**, 1411-1416, doi:10.1016/j.clinph.2010.12.034 (2011).
- 55 Hamada, M., Murase, N., Hasan, A., Balaratnam, M. & Rothwell, J. C. The role of interneuron networks in driving human motor cortical plasticity. *Cerebral cortex* **23**, 1593-1605, doi:10.1093/cercor/bhs147 (2013).
- 56 Esser, S. K. *et al.* A direct demonstration of cortical LTP in humans: a combined TMS/EEG study. *Brain research bulletin* **69**, 86-94, doi:10.1016/j.brainresbull.2005.11.003 (2006).
- 57 Quartarone, A. *et al.* Distinct changes in cortical and spinal excitability following high-frequency repetitive TMS to the human motor cortex. *Experimental brain research* **161**, 114-124, doi:10.1007/s00221-004-2052-5 (2005).
- 58 Stagg, C. J. *et al.* Neurochemical effects of theta burst stimulation as assessed by magnetic resonance spectroscopy. *Journal of neurophysiology* **101**, 2872-2877, doi:10.1152/jn.91060.2008 (2009).
- 59 Baeken, C. *et al.* The impact of HF-rTMS treatment on serotonin(2A) receptors in unipolar melancholic depression. *Brain stimulation* **4**, 104-111, doi:10.1016/j.brs.2010.09.002 (2011).

- 60 Strafella, A. P., Paus, T., Barrett, J. & Dagher, A. Repetitive transcranial magnetic stimulation of the human prefrontal cortex induces dopamine release in the caudate nucleus. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **21**, RC157 (2001).
- 61 Di Lazzaro, V. *et al.* Motor cortex stimulation for amyotrophic lateral sclerosis. Time for a therapeutic trial? *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology* **115**, 1479-1485, doi:10.1016/j.clinph.2004.01.027 (2004).
- 62 Angelucci, F. *et al.* Transcranial magnetic stimulation and BDNF plasma levels in amyotrophic lateral sclerosis. *Neuroreport* **15**, 717-720 (2004).
- 63 Zanette, G., Forgiione, A., Manganotti, P., Fiaschi, A. & Tamburin, S. The effect of repetitive transcranial magnetic stimulation on motor performance, fatigue and quality of life in amyotrophic lateral sclerosis. *Journal of the neurological sciences* **270**, 18-22, doi:10.1016/j.jns.2008.01.011 (2008).
- 64 Di Lazzaro, V. *et al.* Repetitive transcranial magnetic stimulation for ALS. A preliminary controlled study. *Neuroscience letters* **408**, 135-140, doi:10.1016/j.neulet.2006.08.069 (2006).
- 65 Di Lazzaro, V. *et al.* Motor cortex stimulation for ALS: a double blind placebo-controlled study. *Neuroscience letters* **464**, 18-21, doi:10.1016/j.neulet.2009.08.020 (2009).
- 66 Munneke, M. A. *et al.* Cumulative effect of 5 daily sessions of theta burst stimulation on corticospinal excitability in amyotrophic lateral sclerosis. *Muscle & nerve* **48**, 733-738, doi:10.1002/mus.23818 (2013).
- 67 Quartarone, A. *et al.* Motor cortex abnormalities in amyotrophic lateral sclerosis with transcranial direct-current stimulation. *Muscle & nerve* **35**, 620-624, doi:10.1002/mus.20737 (2007).
- 68 Munneke, M. A. *et al.* Transcranial direct current stimulation does not modulate motor cortex excitability in patients with amyotrophic lateral sclerosis. *Muscle & nerve* **44**, 109-114, doi:10.1002/mus.22012 (2011).
- 69 Madhavan S Pt, P., Sivaramakrishnan A Pt, M. S., Bond, S. M. & Jiang, Q. M. Safety and feasibility of transcranial direct current stimulation in amyotrophic lateral sclerosis - a pilot study with a single subject experimental design. *Physiotherapy theory and practice*, 1-6, doi:10.1016/j.clinph.2018.01.061 10.1080/09593985.2018.1443536 (2018).
- 70 Heroux, M. E., Taylor, J. L. & Gandevia, S. C. The Use and Abuse of Transcranial Magnetic Stimulation to Modulate Corticospinal Excitability in Humans. *PloS one* **10**, e0144151, doi:10.1371/journal.pone.0144151 (2015).
- 71 Horvath, J. C., Forte, J. D. & Carter, O. Evidence that transcranial direct current stimulation (tDCS) generates little-to-no reliable neurophysiologic effect beyond MEP amplitude modulation in healthy human subjects: A systematic review. *Neuropsychologia* **66**, 213-236, doi:10.1016/j.neuropsychologia.2014.11.021 (2015).
- 72 Broadbent, H. J. *et al.* Blinding success of rTMS applied to the dorsolateral prefrontal cortex in randomised sham-controlled trials: a systematic review. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry* **12**, 240-248, doi:10.3109/15622975.2010.541281 (2011).
- 73 Brunoni, A. R., Schestatsky, P., Lotufo, P. A., Bensenor, I. M. & Fregni, F. Comparison of blinding effectiveness between sham tDCS and placebo sertraline in a 6-week major depression randomized clinical trial. *Clinical neurophysiology : official*

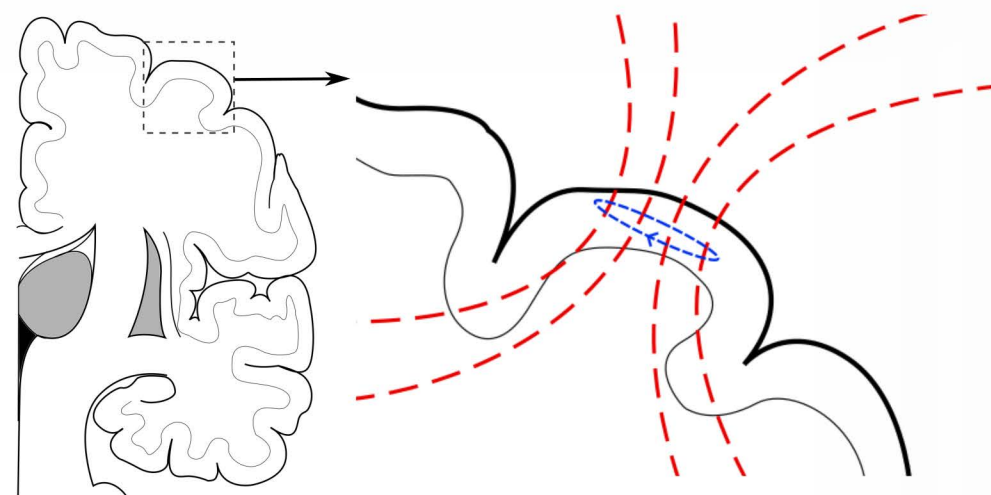
- journal of the International Federation of Clinical Neurophysiology* **125**, 298-305, doi:10.1016/j.clinph.2013.07.020 (2014).
- 74 Caslake, R. Difficulties with control arms in repetitive magnetic stimulation studies. *Journal of neurology, neurosurgery, and psychiatry* **85**, 1182, doi:10.1136/jnnp-2014-307906 (2014).
- 75 Duecker, F. & Sack, A. T. Rethinking the role of sham TMS. *Frontiers in psychology* **6**, 210, doi:10.3389/fpsyg.2015.00210 (2015).
- 76 Bang, H., Ni, L. & Davis, C. E. Assessment of blinding in clinical trials. *Controlled clinical trials* **25**, 143-156, doi:10.1016/j.cct.2003.10.016 (2004).
- 77 Batsikadze, G., Moliadze, V., Paulus, W., Kuo, M. F. & Nitsche, M. A. Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. *The Journal of physiology* **591**, 1987-2000, doi:10.1113/jphysiol.2012.249730 (2013).
- 78 Geevasinga, N. *et al.* Riluzole exerts transient modulating effects on cortical and axonal hyperexcitability in ALS. *Amyotrophic lateral sclerosis & frontotemporal degeneration* **17**, 580-588, doi:10.1080/21678421.2016.1188961 (2016).
- 79 Schwenkreis, P. *et al.* Riluzole suppresses motor cortex facilitation in correlation to its plasma level. A study using transcranial magnetic stimulation. *Experimental brain research* **135**, 293-299 (2000).
- 80 Turner, M. R. & Verstraete, E. What does imaging reveal about the pathology of amyotrophic lateral sclerosis? *Current neurology and neuroscience reports* **15**, 45, doi:10.1007/s11910-015-0569-6 (2015).
- 81 Alekseichuk, I., Turi, Z., Amador de Lara, G., Antal, A. & Paulus, W. Spatial Working Memory in Humans Depends on Theta and High Gamma Synchronization in the Prefrontal Cortex. *Current biology : CB* **26**, 1513-1521, doi:10.1016/j.cub.2016.04.035 (2016).
- 82 Proudfoot, M. *et al.* Increased cerebral functional connectivity in ALS: A resting-state magnetoencephalography study. *Neurology* **90**, e1418-e1424, doi:10.1212/WNL.0000000000005333 (2018).
- 83 Menke, R. A. *et al.* Increased functional connectivity common to symptomatic amyotrophic lateral sclerosis and those at genetic risk. *Journal of neurology, neurosurgery, and psychiatry* **87**, 580-588, doi:10.1136/jnnp-2015-311945 (2016).
- 84 Proudfoot, M., Woolrich, M. W., Nobre, A. C. & Turner, M. R. Magnetoencephalography. *Practical neurology* **14**, 336-343, doi:10.1136/practneurol-2013-000768 (2014).
- 85 Proudfoot, M., Bede, P. & Turner, M. R. Imaging Cerebral Activity in Amyotrophic Lateral Sclerosis. *Frontiers in neurology* **9**, 1148, doi:10.3389/fneur.2018.01148 (2018).
- 86 McMackin, R. *et al.* Measuring network disruption in neurodegenerative diseases: New approaches using signal analysis. *Journal of neurology, neurosurgery, and psychiatry*, doi:10.1136/jnnp-2018-319581 (2019).



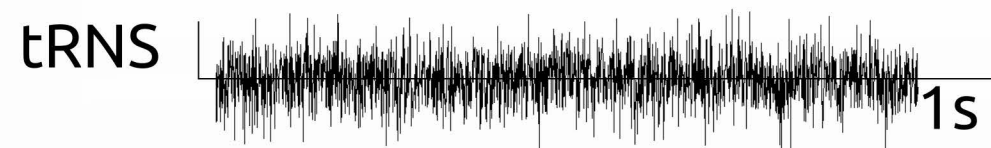
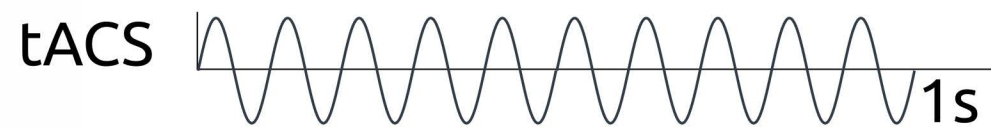
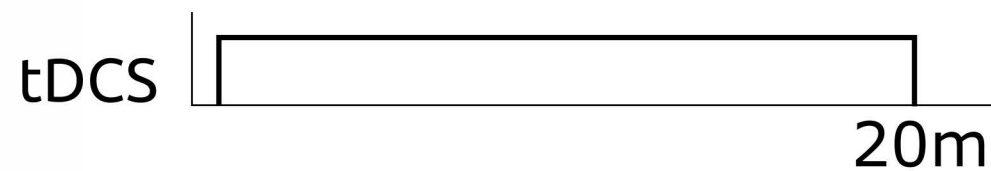
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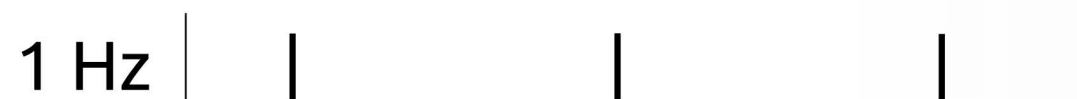


Table 1 - Summary of identified studies using non-invasive brain stimulation techniques in ALS with aim of prolonged neuromodulation.

Transcranial magnetic stimulation

Author / Year	Trial Design	Subjects	Site	Protocol	Schedule	Sham	Riluzole	Inclusion criteria	Exclusion criteria	Outcome measures	Findings
Di Lazzaro 2004	Pilot	4 ALS	Bilateral motor cortex	1 Hz or 20 Hz	Variable	none	2 taking	Definite ALS	Not stated	Norris scale, MRC	Well tolerated. Slower progression in 1 Hz (insufficient power).
Angelucci 2004	Cross sectional	4 ALS 10 healthy	Bilateral motor cortex	1 Hz or 20 Hz	8 days	none	Not stated	Definite ALS	Not stated	Serum BDNF	20 Hz rTMS transiently reduces BDNF in ALS. 1 Hz reduces BDNF in controls but not ALS.
Zanette 2008	RCT	10 ALS 5 active 5 sham	Bilateral motor cortex	5 Hz	5 days per week / 2 weeks	Sham coil	all	Probable/Definite ALS	Not stated	ALSFRS-R, MRC, Grip strength, fatigue, QoL	No effect on ALSFRS-R and MRC. Improved grip strength and QoL at 1 week, not significant at 2 weeks.
Di Lazzaro 2009	RCT	20 ALS 10 active 10 sham	Bilateral motor cortex	cTBS	5 days per month / 12 months	Sham coil	all	Probable/Definite ALS. Age > 18	TMS risk, severe medical condition	ALSFRS-R, MRC, AMT, CMCT	Active rTMS reduced rate of ALSFRS-R decline at 6 months, but insignificant at 12 months. No effect on AMT/CMCT.
Di Lazzaro 2010	Long term pilot	1 ALS	Bilateral motor cortex	cTBS	5 days per month / 26 months	none	all	Definite ALS	Not stated	ALSFRS-R, MEP, CMCT	Well tolerated. Both patients deteriorated.
Munneke 2013	Cross sectional	10 ALS 10 healthy	Left motor cortex	cTBS	5 days	none	all	R handed. Prob/Def ALS. Spinal onset within 6-36 months	Familial ALS, TMS risk	CMAP, MEP, RMT, SICI, ICF	rTMS reduces MEP amplitude and resting motor threshold in both ALS patients and controls.
Di Lazzaro 2014	Open label	3 ALS	Bilateral motor cortex	cTBS	5 days per month / 6 months	none - open label	all	Completed sham arm of Di Lazzaro 2009 trial	TMS risk, severe medical condition	ALSFRS-R, respiratory failure, tracheostomy	rTMS reduces rate of ALSFRS-R decline.
Ceccanti 2018	Cross sectional	24 ALS	Non-dominant motor cortex + median nerve stimulation	0.3 Hz	2 days pre+post riluzole	none	pre+post riluzole	Probable/Definite ALS	Not specified	MEP, RMT, median nerve sensory threshold	Paired associative stimulation increases MEP amplitude in both ALS patients and controls. Effect significantly reduced post-riluzole.

Transcranial direct current stimulation

Study	Study type	Subjects	Electrode montage	Protocol	Schedule	Sham	Riluzole	Inclusion criteria	Exclusion criteria	Outcome measures	Findings
Quartarone 2007	Cross sectional	8 ALS 8 healthy	L motor cortex, R frontal	1 mA anodal or cathodal	2x 7min 1 week apart	none	1 taking	Definite ALS	MEP not elicited	MEP, RMT, AMT, SICI, ICF	After effects of both anodal and cathodal tDCS seen in controls but not in ALS
Munneke 2011	Cross sectional	10 ALS 10 healthy	L motor cortex, R frontal	1 mA cathodal	3x (7,11 or 15min) 1 week apart	none	all	Probable ALS	Not specified	MEP, motor threshold, SICI, ICF	tDCS reduces MEP amplitude in controls but not ALS
Madhavan 2018	Pilot	1 ALS	L motor cortex, R frontal	2mA anodal, cathodal or sham.	12x 20min over 4 weeks. Washout between protocols	30s current ramp	Not specified	Not stated	Not stated	Muscle strength, ALSFRS-R, MEP	No significant effects. MEP could not be evoked before or after stimulation.

Abbreviations: **SICI** - short interval cortical inhibition. **AMT** - active motor threshold. **CMCT** - central motor conduction time. **BDNF** - Brain-derived neurotrophic factor. **MRC** - Medical Research Council power scale. **ALSFRS-R** - ALS functional rating scale (revised). **MEP** - motor evoked potential. **rTMS** - repetitive transcranial magnetic stimulation. **cTBS** - continuous theta burst stimulation. **tDCS** - transcranial direct current stimulation. **QoL** - quality of life. **CMAP** - compound motor action potential